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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/634,740	08/05/2003	Alice Y. Ting	M00656.70097.US	8302
23628	7590	03/01/2005	EXAMINER MONDESI, ROBERT B	
WOLF GREENFIELD & SACKS, PC FEDERAL RESERVE PLAZA 600 ATLANTIC AVENUE BOSTON, MA 02210-2211			ART UNIT 1653	PAPER NUMBER

DATE MAILED: 03/01/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/634,740	TING, ALICE Y.	
	Examiner	Art Unit	
	Robert B Mondesi	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --
Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) Responsive to communication(s) filed on 17 November 2004.
 2a) This action is FINAL. 2b) This action is non-final.
 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) Claim(s) 1-129 is/are pending in the application.
 4a) Of the above claim(s) 1-20,33,37,39 and 46-49 is/are withdrawn from consideration.
 5) Claim(s) _____ is/are allowed.
 6) Claim(s) 21-32,34-36,38 and 40-45 is/are rejected.
 7) Claim(s) _____ is/are objected to.
 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) The specification is objected to by the Examiner.
 10) The drawing(s) filed on 05 August 2003 is/are: a) accepted or b) objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) All b) Some * c) None of:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)	4) <input type="checkbox"/> Interview Summary (PTO-413)
2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)	Paper No(s)/Mail Date. _____ .
3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date	5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152)
	6) <input type="checkbox"/> Other: _____ .

DETAILED ACTION

Applicants' election of Invention Group III, Claims 21-45, and further election of patentably distinct invention designated as SEQ ID No: 18 in response to the restriction requirement mailed November 17, 2004 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 1-129 are pending. **Claims 1-20, 33 (drawn to non-elected patentably distinct product SEQ ID No: 3), 37 (drawn to non-elected patentably distinct product SEQ ID No: 1), 39 (drawn to non-elected patentably distinct product SEQ ID No: 2) and 46-129** are withdrawn. **Claims 21-32, 34-36, 38 and 40-45** are presently under examination.

Priority

The current application filed on August 05, 2003 claims priority to provisional application 60/425,578 filed on November 12, 2002.

Preliminary Amendment

The preliminary amendment filed October 20, 2003 has been entered.

Information Disclosure Statement

The IDS(s) filed May 24, 2004 and September 27, 2004 have been received and are signed and considered, a copy of the PTO 1449 is attached to the following document.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 21-32, 34-36, 38 and 40-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 21 cites a method of determining the level of histone modification in a biological sample comprising contacting a biological sample with a fusion protein reporter comprising a core comprising a histone modification-specific binding domain conjugated to a histone polypeptide; however the applicants do not explain the end result of this method. It is not clear as to whether the contacting a biological sample with the said polypeptide is designed to have an inhibitory effect or a non-inhibitory effect. Furthermore the step of monitoring the level of fluorescence resonance energy transfer (FRET) in the biological sample, is not an actual step. There is no stated end result to this step nor is it involved in a cause-effect process step. **Claims 22-32, 34-36, 38 and 40-45** are dependent claims that do not further clarify the independent claim that they depend from.

In **claim 32**, FHA and WW are made up abbreviations for polypeptide domains, applicants need to refer to the residues in the amino acid sequence of the mentioned domains.

In **claim 34**, Gcn5, TAF α 250, P/CAF, CBP, BRG1, Swi2, and Sth1 are made up abbreviations for peptides and polypeptide domains, applicants need to refer to the residues in the amino acid sequence of the mentioned domains.

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In **claim 35**, HP1, MRG15, MRG-1, cynCDY, Hrp3, dMi-2, CHD5, Swi6, are made up abbreviations for peptides and polypeptide domains, applicants need to refer to the residues in the amino acid sequence of the mentioned domains.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 21-32, 34-36 40-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 01/33199 (cited in the IDS filed May 24, 2004) in view of Akhtar et al.

The elected invention of the present application is a method of using fusion reporters for monitoring of post-translational modifications, such as acylation, methylation and phosphorylation, of proteins in cells. This modification is detected by FRET which involves contacting a biological sample with a fusion protein reporter of any of the forgoing embodiments, determining a first level of fluorescence resonance energy transfer (FRET) in the biological sample, determining a second subsequent level of FRET in the biological sample, and comparing the first and second levels of FRET as a measure of the change in the level of protein modification in the biological sample.

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WO 01/33199 teaches that when a fluorescent molecule absorbs light, an electron is excited to a higher energy level and typically the electron loses some energy before decaying back to the ground state. During this transition, a photon is emitted with less energy than the excitation photon and hence a longer wavelength. If a second fluorophor is in close proximity, the energy released by the electron as it decays in the donor fluorophor may be transferred directly to the second acceptor fluorophore and excite one of the electrons of the latter to a higher energy level. When the electron in the acceptor in the acceptor decays from this state, an even longer wavelength is released and this process is termed fluorescence resonance energy transfer (FRET) (page 1, lines 14-22).

WO 01/33199 teaches further that the inventions uses a target binding site which is attached to a first fluorescent polypeptide, a mimic peptide which is capable of binding to the target binding site peptide and is attached to a second fluorescent polypeptide and a linker which connects the two fluorescent polypeptides and which allows the distance between said fluorescent polypeptides to vary, said fluorescent polypeptides being such as to display fluorescence resonance energy transfer (FRET) between them (page 2, lines 15-23).

WO 01/33199 states that suitable fluorescent polypeptides mentioned above include those from the Green fluorescent protein (GFP) family of polypeptides and several classes of useful GFP mutants have been disclosed including : (1) red shifted GFP; (2) blue fluorescent protein (BFP); cyan fluorescent protein (CFP), and yellow fluorescent protein (YFP) (page 8, lines 23-39).

WO 01/33199 also teaches that the use of the construct of the invention in living cells falls into two main classes: (i) use in isolated cells in culture and (ii) use in intact multi-cellular organisms. Isolated cells in culture may be microbial for example , bacterial, fungal, plant or animal and the construct of the invention could be any substance or substances including peptides such as posttranslational modification sites for example phosphorylation sites (page 22, lines 5-15).

On page 15 of the specification the applicants state that examples of modification specific binding polypeptides for detecting phosphorylation modification are known in the art as evidenced by Fu et al. and Aitken et al. (14-3-3 domains) and Yaffe et al. (FHA or WW domains). The applicants state further that examples of modification-specific binding polypeptides for detecting protein acetylation include bromodomains (GCN5, P/CAF, CBP, BRG1, Swi2) are known in the art as evidenced by the following publications *FEBS Lett* 513 (1): 124-8 (2002), *Front Biosci* 6:D1019-23 (2001) and *Nat Struct Biol* 6(7):601-4 (1999)); and examples of modification-specific binding polypeptides for detecting methylation include chromo-domains (HP1, MRG-5, CHD5 and Swi6) which are also well known in the art as evidenced by *nature* 407(6802):405-9 (2000).

WO 01/33199 does not teach that the stated method above comprises fusion proteins comprising a histone modification specific binding domain wherein the histone polypeptide that is acetylated is a H4 histone polypeptide.

Akhtar et al. disclose a histone polypeptide that is H4 polypeptide and is acetylated at lysine 16 by the histone acetyltransferase.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a H4 histone polypeptide in a fusion protein reporter construct as a histone modification specific binding domain in a method of determining the level of histone modification in a biological sample using FRET for the advantages of an acetylation reporter as taught by Aktar et al. and WO 01/33199, see Aktar et al. page 408 and WO 01/33199 page 22, lines 5-22).

Claims 21-32, 34-36 40-45 are rejected under 35 U.S.C. 103(a) as being obvious over Tsien et al. United States Patent Application Publication US2003/0186229 in view of Akhtar et al.

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). For applications filed on or after November 29, 1999, this rejection might also be overcome by showing that the subject matter of the

reference and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person. See MPEP § 706.02(I)(1) and § 706.02(I)(2).

Tsien et al. teach that their invention relates to a chimeric phosphorylation indicator, which contains, in operative linkage, a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule, wherein the phosphoaminoacid binding domain specifically binds to a phosphoaminoacid when present in the phosphorylatable domain, the donor molecule and the acceptor molecule exhibit a detectable resonance energy transfer when the donor is excited, and the phosphorylatable domain and phosphoaminoacid binding domain do not substantially emit light to excite the acceptor. The donor molecule or the acceptor or both can be a fluorescent protein, or a luminescent molecule, or a combination thereof. In one embodiment, each of the donor molecule and the acceptor molecule is a fluorescent protein.

Tsien et al. also teach that chimeric phosphorylation indicator of the invention contains a fluorescent protein donor molecule, resonance energy transfer can be detected as fluorescence resonance energy transfer (FRET). Where the donor molecule is a luminescent molecule, resonance energy transfer is detected as luminescent resonance energy transfer) and depending on the particular structure of the chimeric phosphorylation indicator as disclosed herein, FRET or LRET can be increased or decreased due to phosphorylation of the indicator by a kinase, and, likewise, can be increased or decreased due to phosphorylation of the

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indicator by a phosphatase. A change in FRET or LRET can be determined by monitoring the emission spectrum of the acceptor.

Tsien et al. teach further that a fluorescent protein in a chimeric phosphorylation indicator can be a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP, including a non-oligomerizing fluorescent protein and a GFP useful in a chimeric phosphorylation indicator can be an Aequorea GFP, a Renilla GFP, a Phialidium GFP, or a fluorescent protein related to an Aequorea GFP, a Renilla GFP, or a Phialidium GFP. A fluorescent protein related to an Aequorea GFP, for example, can be a cyan fluorescent protein (CFP), or a yellow fluorescent protein (YFP), or a spectral variant of the CFP or YFP (section 010-015)

Tsien et al. teach n one embodiment, the specific amino acid that can be phosphorylated by a kinase in the phosphorylatable domain of a chimeric phosphorylation indicator is not phosphorylated, such that the indicator can be used to detect the presence of the kinase in a sample. In another embodiment, the specific amino acid that can be phosphorylated by a kinase in the phosphorylatable domain of a chimeric phosphorylation indicator is phosphorylated, such that the indicator can be used to detect the presence of a phosphatase in a sample. The specific amino acid can be any amino acid that can be phosphorylated by a kinase or dephosphorylated by a phosphatase, for example, serine, threonine, tyrosine, or a combination thereof and furthermore the invention also relates to a chimeric phosphorylation indicator, which contains a phosphorylatable polypeptide and a fluorescent protein wherein the specific amino acid that can be phosphorylated by a

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kinase in the phosphorylatable polypeptide can be unphosphorylated, such that the indicator can be used to detect a kinase activity, or can be phosphorylated, such that the indicator can be used to detect a phosphatase activity (page 2 ,sections [0018] and [0019]).

Tsien et al. also teach that of a chimeric phosphorylation indicator containing a phosphorylatable polypeptide and a fluorescent protein, the phosphorylatable polypeptide comprises an N-terminal portion and a C-terminal portion, and the fluorescent protein is operatively inserted between the N-terminal portion and C-terminal portion of the phosphorylatable polypeptide. The fluorescent protein can be any fluorescent protein, for example, a GFP, an RFP, or a fluorescent protein related to a GFP or an RFP, and can be in a circularly permuted form. The phosphorylatable polypeptide can be any substrate for a kinase, for example, a tyrosine kinase or a serine/threonine kinase, or for a phosphatase. The fluorescent protein can be operatively inserted into any region of the phosphorylatable polypeptide, for example, in a hinge region or a turn, provided the ability of the polypeptide to act as a substrate is not disrupted (page 2, section [0020]).

Tsien et al. teach a method for detecting a kinase or phosphatase in a sample is performed by contacting the sample with a chimeric phosphorylatable indicator containing a phosphorylatable polypeptide and a fluorescent protein, determining a fluorescence property in the sample, wherein the presence of kinase or phosphatase activity in the sample results in a change in the

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fluorescence property as compared to the fluorescent property in the absence of a kinase or phosphatase activity, thereby detecting the kinase or phosphatase in the sample (page 4, section 027).

Tsien et al. teach further that their invention also relates to a method for detecting a kinase inhibitor or phosphatase inhibitor. Such a method can be performed, for example, by determining a first fluorescence property of a chimeric phosphorylatable indicator in the presence of a kinase or a phosphatase, contacting the chimeric phosphorylatable indicator with a composition suspected of being a kinase inhibitor or a phosphatase inhibitor, determining a second fluorescence property of a chimeric phosphorylatable indicator in the presence of the composition, wherein a difference in the first fluorescence property and second fluorescence property identifies the composition as a kinase inhibitor or phosphatase inhibitor (page 4, section 029).

On page 15 of the specification the applicants state that examples of modification specific binding polypeptides for detecting phosphorylation modification are known in the art as evidenced by Fu et al. and Aitken et al. (14-3-3 domains) and Yaffe et al. (FHA or WW domains). The applicants state further that examples of modification-specific binding polypeptides for detecting protein acetylation include bromodomains (GCN5, P/CAF, CBP, BRG1, Swi2) are known in the art as evidenced by the following publications *FEBS Lett* 513 (1): 124-8 (2002), *Front Biosci* 6:D1019-23 (2001) and *Nat Struct Biol* 6(7):601-4 (1999)); and examples of modification-specific binding polypeptides for detecting methylation include chromo-domains (HP1, MRG-5, CHD5

and Swi6) which are also well known in the art as evidenced by *nature* 407(6802):405-9 (2000).

Tsien et al. do not teach that the stated method above comprises fusion proteins comprising a histone modification specific binding domain wherein the histone polypeptide that is acetylated is a H4 histone polypeptide.

Akhtar et al. disclose a histone polypeptide that is H4 polypeptide and is acetylated at lysine 16 by the histone acetyltransferase.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a H4 histone polypeptide in a fusion protein reporter construct as a histone modification specific binding domain in a method of determining the level of histone modification in a biological sample using FRET for the advantages of an acetylation reporter as taught by Aktar et al. and WO 01/33199, see Aktar et al. page 408 and WO 01/33199 page 22, lines 5-22).

Claims 21, 26-28, 31-32, 35-36, 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 01/33199 in view Agalioti (cited in the IDS filed May 24, 2004).

WO 01/33199 teaches a method of determining the level of modification in a biological sample as mentioned above.

WO 01/33199 does not teach that the above mentioned method comprises fusion proteins comprising a histone modification specific binding domain wherein the histone polypeptide that is acetylated is a H4 histone polypeptide.

Agalioti et al. disclose a histone polypeptide that is H4 polypeptide and is acetylated at lysine 16 by the histone acetyltransferase (Results, pages 382-385).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a H4 histone polypeptide in a fusion protein reporter construct as a histone modification specific binding domain in a method of determining the level of histone modification in a biological sample using FRET for the advantages of an acetylation reporter as taught by Agalioti et al. and WO 01/33199, see Agalioti et al. pages 388-389 and WO 01/33199 page 22, lines 5-22).

Claims 21, 26-28, 31-32, 35-36, 38 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 01/33199 in view of Reichheld et al.

WO 01/33199 teaches a method of determining the level of modification in a biological sample as mentioned above.

WO 01/33199 does not teach that the above mentioned method comprises fusion proteins comprising a histone modification specific binding domain wherein the histone polypeptide that is an H3 polypeptide comprising the amino acid sequence set forth as SEQ ID No:18.

Reichheld et al. disclose a histone polypeptide that is an H3 polypeptide comprising the amino acid sequence set forth as SEQ ID No:18 (figure 4, page 3259).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a H3 polypeptide comprising the amino acid sequence set forth as SEQ ID No:18 in a fusion protein reporter construct as a histone modification specific binding domain in a method of determining the level of histone modification in a

biological sample using FRET for the advantages of an acetylation reporter as taught by Reichheld et al. and WO 01/33199, see Reichheld et al. pages 3260-3261 and WO 01/33199 page 22, lines 5-22).

Conclusion

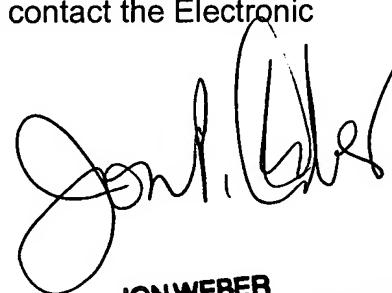
No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Robert B Mondesi whose telephone number is 571-272-0956. The examiner can normally be reached on 9am-5pm, Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jon Weber can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).


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